METHODS

Subjects

Atopic asthmatic and age-matched healthy children without asthma, 6 to 16 years of age, who were undergoing an elective surgical procedure that required endotracheal intubation and general anesthesia were recruited. Children with asthma (n = 12; mean age, 12.2 year) had at least a 1-year history of physician-diagnosed asthma, used albuterol at least twice a month or were taking a daily inhaled corticosteroid or leukotriene receptor antagonist, and were born at ≥36 weeks of gestation. Healthy subjects (n = 9; mean age, 11.4 years) were born at ≥36 weeks of gestation; had no history of asthma, reactive airway disease, chronic daily cough, or physician-diagnosed obstructive lung disease; and had no history of prior treatment with a systemic or inhaled corticosteroid, albuterol, or oxygen (see Table E1). Children with asthma had a history of one or more of the following atopic features: positive skin prick test or RAST for a common aeroallergen, elevated serum IgE (>100 IU/mL), physician-diagnosed allergic rhinitis, or physician-diagnosed atopic dermatitis. Healthy subjects lacked a history of any of these atopic features. Written consent was obtained from parents of subjects. The study was approved by the Seattle Children's Hospital Institutional Review Board.

Epithelial cell isolation

Immediately after the endotracheal tube was secured 3 BEC samples were obtained from subjects while under general anesthesia with the use of 4-mm Harrell unsheathed bronchoscope cytology brushes (Conmed Corporation, Utica, NY). As described by Lane et al,[E1] the unprotected brush was inserted through an endotracheal tube, advanced until resistance was felt, and rubbed against the airway surface for 2 seconds. Cells were seeded onto T-25 cell culture flasks precoated with type I collagen. Cultures were maintained at 37°C in an atmosphere of 5% CO_2 in a humidified incubator. Cells were cultured in BEGM (Clonetics BEGM; Lonza, Basel, Switzerland) containing gentamicin and amphotericin B and were further supplemented with penicillin/streptomycin (100 μg/mL; Invitrogen). Fluconazole (25 μg/mL) was added to primary cultures for the first 96 hours, after which medium was aspirated and replaced with BEGM without fluconazole. BEGM was thereafter changed every 48 hours until the culture reached ~70% to 90% confluence. When P0 flasks became 70% to 90% confluent, cells were passaged into 3 new passage 1 (P1) T25 flasks.

ALI epithelial cell cultures

For ALI cultures, Corning Costar 12-mm 0.4-μm Transwells (Corning Life Sciences, Tewksbury, Mass) were precoated with type I collagen, then seeded with P2 epithelial cells at a concentration of 100,000 cells per transwell. All seeded cells screened negative for evidence of mycoplasma infection (Mycoprobe Mycoplasma Kit; R&D Systems, Minneapolis, Minn). Cells were grown submerged in BEGM until 100% confluence, at which time apical medium was removed, and basolateral medium was replaced with ALI medium. ALI medium consisted of a 1:1 mixture of BEGM and DMEM supplemented with *all-trans* retinoic acid (30 ng/mL), human recombinant epidermal growth factor (0.5 ng/mL), $MgCl_2$ (0.6 mmol/L), $CaCl_2$ (1 mmol/L), and penicillin/streptomycin (100 μg/mL). ALI medium in the basolateral compartment was changed every other day, and cells were differentiated at an ALI for 21 days before initiation of cell culture experiments. Differentiated BECs were exposed at the apical surface of transwells with RSV A2 strain at a MOI of 0.5 or an equivalent volume of control Vero cell supernatant fluid for 2 hours. Sampling of basolateral supernatant fluid was performed 96 hours after RSV or control exposure. Each experimental condition per cell line consisted of triplicate transwells.

Subject clinical characterization

A blood sample was drawn and used to measure total serum IgE and RAST allergen-specific IgE to dust mites, cat epithelium, dog epithelium, *Alternaria tenuis*, *Aspergillus fumigatus*, and timothy grass. The fraction of exhaled nitric oxide was measured according to ATS guidelines with the use of a NIOX chemiluminescent nitric oxide analyzer (Aerocrine, Solna, Sweden). Forced vital capacity, FEV_1, and forced expiratory flow at 25% to 75% of forced vital capacity were measured according to ATS guidelines with the use of a VMAX series 2130 spirometer (VIASYS Healthcare, Hong Kong). Spirometry was repeated 15 minutes after administration of 2 puffs of albuterol in children with asthma.

REFERENCE

E1. Lane C, Burgess S, Kicic A, Knight D, Stick S. The use of non-bronchoscopic brushings to study the paediatric airway. Respir Res 2005;6:53.

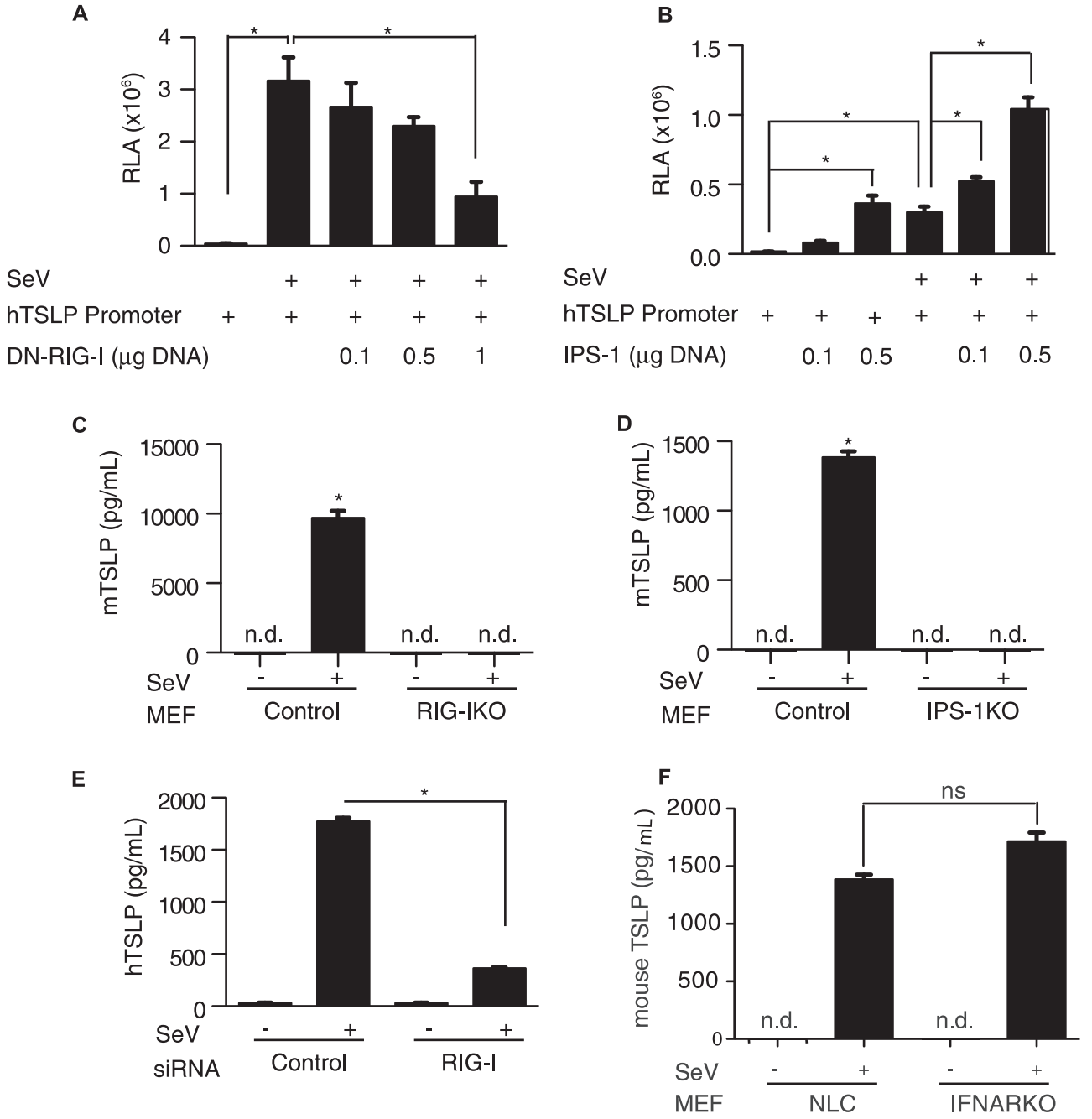

FIG E1. RIG-I and IPS-1 activation mediate SeV-induced TSLP expression. **A** and **B,** A549 cells were transiently transfected with control plasmid or promoter-luciferase constructs that contained human TSLP promoter plasmid alone or in combination with expression vectors for a DN-RIG-I (0.1, 0.5, or 1 μg) (Fig E1, *A*) or IPS-1 (0.1 or 0.5 μg) (Fig E1, *B*). Luciferase activity in the whole cell lysate was normalized with β-galactosidase activity. Data are the means ± SDs of triplicate data points from a representative experiment of 5 independent experiments. **C** and **D,** MEFs from WT, RIG-IKO (Fig E1, *C*) or IPS-1KO (Fig E1, *D*) mice were infected with SeV (100 HAU/mL), and TSLP protein was measured in supernatant fluids after 24 hours. **E,** NHBECs were transiently transfected with control or RIG-I siRNA. After 30 hours, cells were infected with SeV (50 HAU/mL). After 24 hours hTSLP levels in the supernatant fluid were measured by ELISA. **F,** WT or IFNARKO MEFs were infected with SeV (100 HAU/mL) and analyzed for TSLP protein in supernatant fluids by ELISA after 24 hours (n ≥3 independent experiments in all cases). *$P \leq .05$.

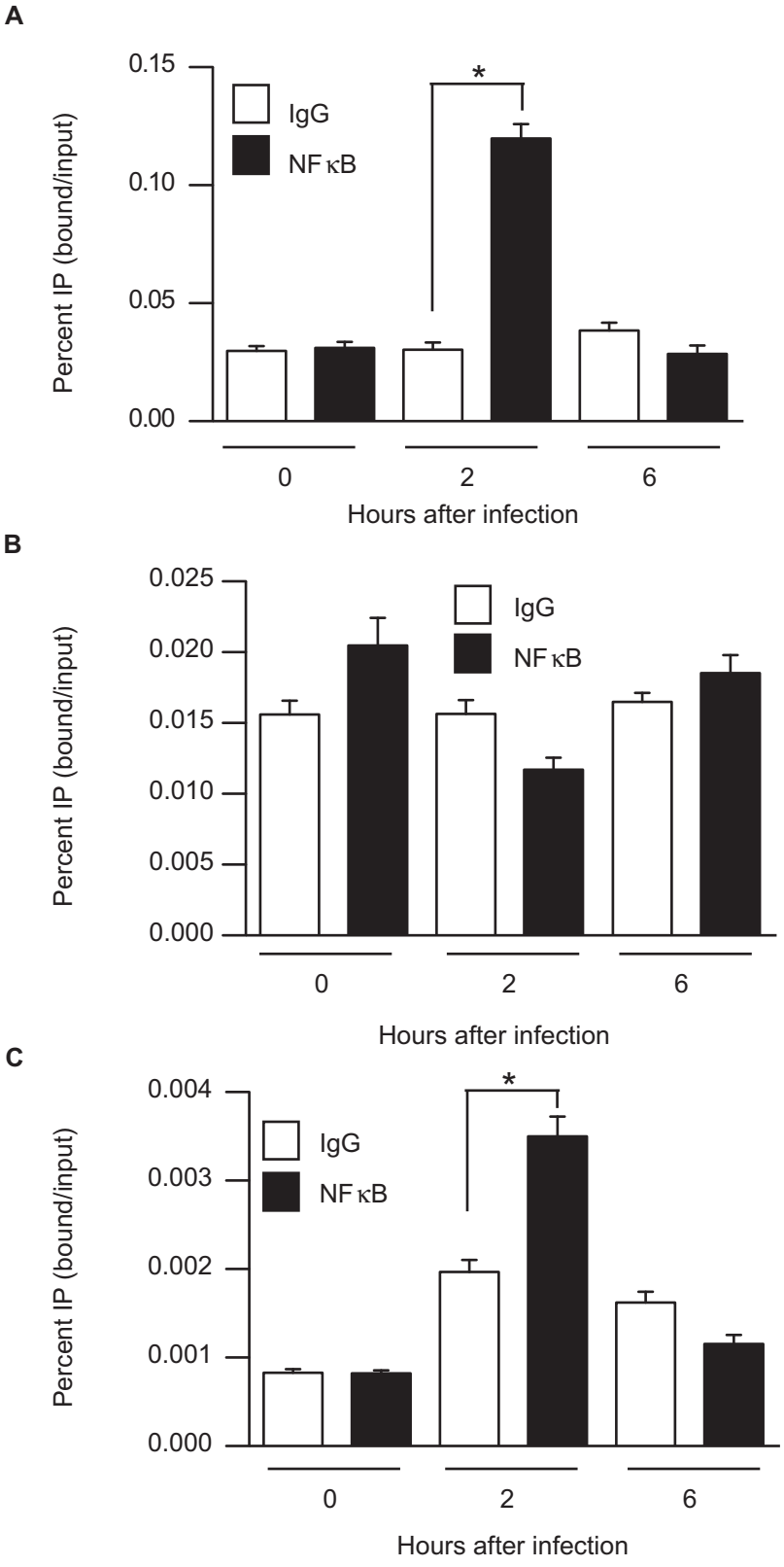

FIG E2. NF-κB is recruited to the endogenous TSLP promoter in response to SeV infection. **A-C,** NHBECs were infected with SeV (50 HAU/mL) for the indicated time course. Soluble chromatin preparation was immunoprecipitated with normal IgG or anti–NF-κB. Purified ChIP and input DNA were analyzed by real-time quantitative PCR with the primers, against the −3.2 (Fig E2, *A*), −1.3 (Fig E2, *B*), and −0.2 (Fig E2, *C*) kb TSLP promoter NF-κB sites, respectively. The amount of ChIP DNA was normalized to that of input DNA. Data are the means ± SDs of triplicate data points from a representative experiment. $*P \leq .05$.

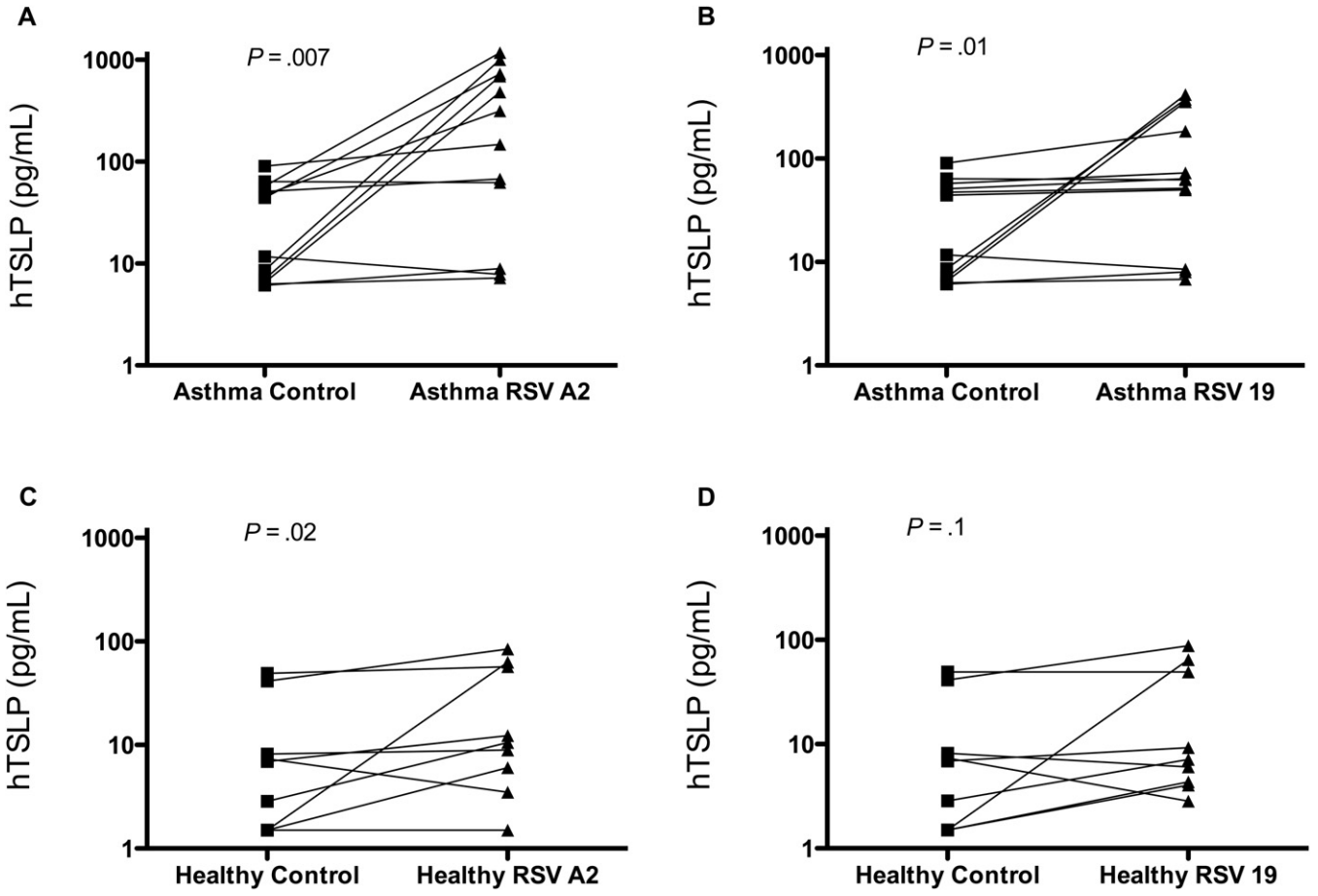

FIG E3. TSLP production increased in response to RSV infection in most individual asthmatic **(A** and **B)** and healthy **(C** and **D)** human epithelial cell cultures; however, the magnitude of increase was greater by cultures from asthmatic children. Data show TSLP protein levels, measured by ELISA, in basolateral culture supernatant fluids from uninfected control cultures and after infection with either RSV A2 or Line 19 at a MOI of 0.5 (n = 12 asthmatic and n = 9 healthy cell lines). *P* values were calculated with the Wilcoxon signed-rank test for paired samples.

TABLE E1. Subject characteristics

	Healthy controls (n = 9)	Patients with asthma (n = 12)	*P* value
Age (y), mean ± SD	10.9 ± 4.6	11.3 ± 3.8	.7
Female sex (%)	44	42	.7
Current use of inhaled steroids, no. (%)	NA	7 (58)	
IgE IU/mL, mean ± SD	23.1 ± 21.5	341 ± 289	.06
FVC (% predicted), mean ± SD	105 ± 8.5	103 ± 14.2	.8
FEV_1/FVC ratio, mean ± SD	0.93 ± 0.06	0.81 ± 0.08	.01
FEV_1 (% predicted), mean ± SD	104 ± 12.4	95 ± 16.5	.2
FEF_{25-75} (% predicted), mean ± SD	98 ± 20.3	82.1 ± 22.5	.2
BDR, no. (%)	NA	6 (50)	
F_{ENO} (ppb), mean ± SD	13.2 ± 17.2	29.6 ± 41.7	.2

BDR, Bronchodilator responsiveness (defined as an increase of ≥12% in FEV_1 or ≥25% in FEF_{25-75} after 2 puffs of albuterol via a metered dose inhaler); *FEF_{25-75}*, forced expiratory flow between 25% and 75% of expiration; *F_{ENO}*, fractional exhaled nitric oxide; *FVC*, forced vital capacity; *NA*, not applicable; *ppb*, part per billion.